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Effects of Chlorpyrifos on Field- and Laboratory-Developed Estuarine Benthic Communities

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16. ABSTRACT

Macrobenthic animal communities, developed in sand-filled aquaria in the laboratory and in the field, were exposed to various concentrations of the insecticide, chlorpyrifos, and effects on community structure assessed. Laboratory communities were continuously exposed to the toxicant for eight weeks during colonization by planktonic larvae in unfiltered Santa Rosa Sound, FL, seawater. Field communities developed for eight weeks in aquaria placed in Santa Rosa Sound and then removed to the laboratory for exposure to chlorpyrifos for one week. Abundance of arthropods was significantly diminished (α=0.05) by measured concentrations of chlorpyrifos >0.1 µg/ℓ in water in laboratory communities and by 5.9 µg/ℓ in water in field communities. Numbers of annelids and chordates in contaminated aquaria were not reduced by the highest concentrations of chlorpyrifos tested, 8.5 µg/ℓ in laboratory-colonized aquaria, and 5.9 µg/ℓ in field-colonized aquaria. One species of annelid, Cistenides gouldii, was more abundant in field aquaria receiving 1.0 µg/ℓ or 5.9 µg/ℓ than in the control and lowest concentration. Molluscan larvae colonizing laboratory aquaria were sensitive to ≥ 0.1 µg/ℓ; however, later developmental stages characterizing field aquaria were not sensitive to ≥ 5.9 µg/ℓ. Although only 20 of 78 animal species appeared in both laboratory and field communities, sensitivity of animals in these tests and in single species tests could be compared.

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EFFECTS OF CHLORPYRIFOS ON FIELD- AND LABORATORY-DEVELOPED ESTUARINE BENTHIC COMMUNITIES

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Macrobenthic animal communities, developed in sand-filled aquaria in the laboratory and in the field, were exposed to various concentrations of the insecticide chlorpyrifos, and effects on community structure were assessed. Laboratory communities were continuously exposed to the toxicant for 8 wk during colonization by planktonic larvae in unfiltered Santa Rosa Sound, Fla., seawater. Field communites were developed for 8 wk in aquaria placed in Santa Rosa Sound, then removed to the laboratory for exposure to chlorpyrifos for one week. Abundance of arthropods was significantly diminished ($\alpha = 0.05$) by measured concentration of chlorpyrifos $\geq$ 0.1 µg/l in water in laboratory communities and by 5.9 µg/l in water in field communities. Numbers of annelids and chordates in contaminated aquaria were not reduced by the highest concentrations of chlorpyrifos tested, 8.5 µg/l in laboratory-colonized aquaria and 5.9 µg/l in field-colonized aquaria. One species of annelid, Cistenides gouldii, *was more abundant in field aquaria receiving 1.0 µg/l or 5.9 µg/l than in the control and lowest concentration. Molluscan larvae colonizing laboratory aquaria were sensitive to $\geq$ 0.1 µg/l; however, later developmental stages characterizing field aquaria were not sensitive to $\leq$ 5.9 µg/l. Although only 20 of 78 animal species appeared in both laboratory and field communities, sensitivity of animals in these tests and in single species tests could be compared.*

INTRODUCTION

Chlorpyrifos [O,O-diethyl O-(3,5,6-trichloro-2-pyridyl)phosphorothioate] has been extensively used as an insecticide for more than 10 years. Some of its environmental properties include a solubility in seawater of 73 µg/l, a half-life in sediment-water laboratory systems of 24 d, and an octano-water partition coefficient (log *P*) value of 5.2 (Schimmel et al., 1982).

Acute toxicity tests conducted at the Gulf Breeze Environmental Research Laboratory (ERL) indicated that chlorpyrifos is highly toxic to some estuarine organisms. Acute toxicity (48- or 96-h LC50) in flowing

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seawater to six fishes (*Cyprinodon variegatus, Fundulus similis, Menidia menidia, Mugil cephalus, Leiostomus xanthurus,* and *Opsanus beta*) ranged from 1.7 to 136 μg/l; that for five crustaceans (*Callinectes sapidus, Palaemonetes pugio, Penaeus duorarum, Penaeus aztecus,* and *Mysidopsis bahia*) ranged from 0.035 to 5.2 μg/l (J. I. Lowe, Gulf Breeze ERL, personal communication; Schimmel et al., 1982). A 96-h exposure of oysters, *Crassostrea virginica,* to 34 μg chlorpyrifos/l flowing seawater caused a 50% decrease in shell deposition (J. I. Lowe, personal communication). Oyster larvae, however, were less sensitive, having a 48-h EC50 for abnormal development of 1991 μg/l in a static test (Borthwick and Walsh, 1981).

To obtain additional information on the effects of chlorpyrifos on the estuarine environment, we conducted studies on the responses of laboratory- and field-colonized benthic communities exposed to this toxicant. Laboratory communities were continuously exposed during development; field communities were exposed after communities had already been established.

METHODS

Laboratory-Colonized Communities

The effect of chlorpyrifos on colonization was determined in the laboratory by comparing numbers and species of estuarine animals that grew from planktonic larvae in aquaria treated with chlorpyrifos for 8 wk (June 16 to August 11, 1980) with those of animals that grew in untreated aquaria. Larvae entered the aquaria as plankton in continuously supplied unfiltered seawater. We used four acrylic plastic apparatuses (Fig. 1), each consisting of a central constant-head box positioned atop six aquaria (40 cm long, 10 cm wide, and 12 cm high). Aquaria were filled to a depth of 5 cm with clean silica sand (particle size 0.2 to 0.8 mm) dredged at least a year previously from Santa Rosa Sound, Fla. Water level in aquaria was maintained at 3 cm above the sand.

Seawater with its constituent plankton was pumped from Santa Rosa Sound to a splitter box where four adjacent glass tubes supplied water at a rate of 1.4 l/min to the four constant-head boxes that routed water to the aquaria (Fig. 1). Flow to each aquarium was maintained at 200 ml/min by adjusting the height of a glass standpipe (2.2 mm ID) in the constant-head box. Water flowed from each aquarium through a notched end-opening; large predators, such as crabs, escaped through these openings before they could affect community structure. Salinity and temperature of incoming seawater were recorded continuously and averaged 26.5 parts per mil (14.5 to 34.0 parts per mil) and 28°C (24 to 31°C). Photoperiod was 12 h.

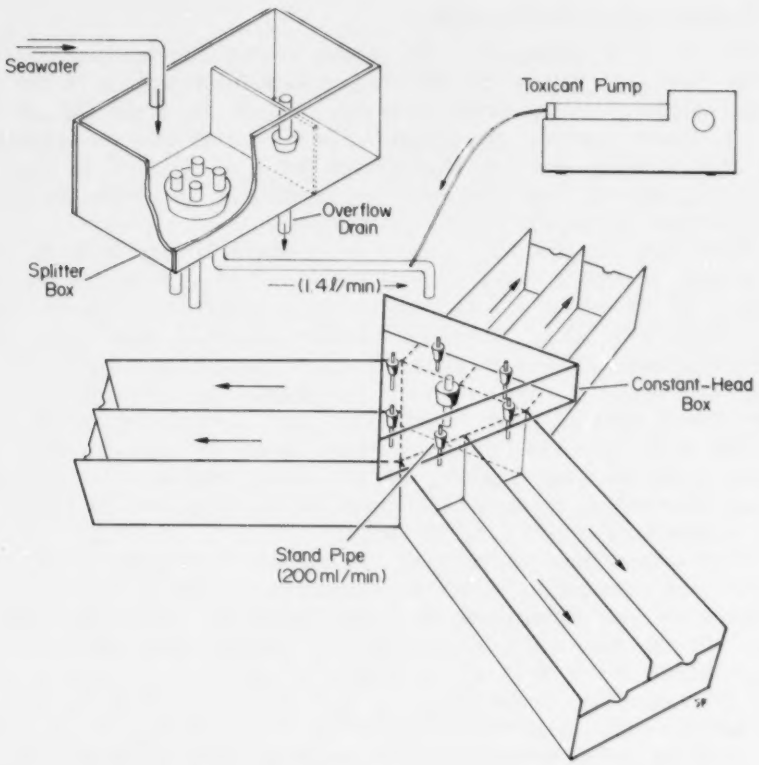

FIGURE 1. One of four identical apparatuses of six aquaria, extending from the splitter box shown, used to test the effect of chlorpyrifos on development of estuarine macrobenthic communities in the laboratory.

Technical-grade chlorpyrifos (92%), dissolved in a stock solution consisting of 15% acetone and 85% triethylene glycol, was metered by syringe pump into seawater entering the center of the constant-head box of each apparatus receiving chlorpyrifos. An equal amount of carrier solvent (10 ml/day, 5 μl/l) was metered into the control apparatus. Nominal concentrations of chlorpyrifos in seawater were 0.1, 1.0, and 10.0 μg/l. Samples of water were taken from constant-head boxes once a week for chemical analyses for chlorpyrifos concentration. Data are presented in measured concentrations.

After 8 wk, animals were collected by siphoning the contents of the aquaria into a 1-mm mesh sieve, after which they were preserved and identified.

Field-Colonized Communities

The effect of chlorpyrifos on benthic animal communities colonized in the field was determined by placing sand-filled aquaria in the field to be colonized by naturally occurring animals for 8 wk (March 9 to May 4, 1981), bringing the established communities into the laboratory, and then exposing them to chlorpyrifos for 1 wk (May 5 to May 12). Following removal and identification, abundance of living animals in contaminated and control aquaria was compared.

Thirty-two aquaria were placed by SCUBA divers in 3 m water at a site in Santa Rosa Sound adjacent to our laboratory. The aquaria, 32 × 32 × 6 cm deep, were constructed of acrylic plastic and filled with sand from the source described previously. They were numbered for identification and grouped by fours (in the form of squares). The aquaria, positioned in the substratum so that their surfaces were level with the surrounding sand, were covered with $\frac{1}{4}$-in (6.4-mm) hardware cloth to exclude large predators, such as crabs. Salinity of the water entering our laboratory during colonization averaged 27.5 parts per mil (18.0 to 32.5 parts per mil); temperature, 20.5°C (15.5 to 26.0°C).

After colonization in the field, the aquaria were collected by divers and covered with acrylic plastic for immediate transfer to the laboratory. Temperature and salinity in field and laboratory at transfer did not differ. In the laboratory, groups of four aquaria were placed in eight acrylic plastic boxes that, when filled, resulted in a 3-cm layer of water over the sand. Two boxes served as eight replicate aquaria for the control and for each of the three chlorpyrifos exposures (0.1, 1.0, and 10.0 μg/l, nominal concentrations). Each group of eight replicates contained an aquarium that had been randomly selected from each of the eight field groups of four aquaria.

Unfiltered seawater was delivered continuously at 2 l/min to the center of each box, and water flowed from the box through notched openings on all sides. Plankters in the seawater could serve as food during exposure.

Chlorpyrifos was metered by syringe pump into seawater entering the center of each box receiving chlorpyrifos. Aquaria used as the control received the same amount of carrier solvent (10 ml/day, 3.5 μl/l) as the aquaria receiving toxicant. Water samples were taken from each box after 1, 3, and 6 d for determination of chlorpyrifos concentrations. Data are presented using measured concentrations. Salinity during exposure averaged 29.0 parts per mil (27.0 to 31.5 parts per mil) and temperature averaged 24°C (23 to 26°C).

After 1 wk of exposure to the toxicant, the communities were harvested. Animals retained by a 1-mm mesh sieve were preserved and identified.

Statistical and Chemical Analyses

Results are presented as pooled data from each chlorpyrifos concentration and the control. One-way analysis of variance (*SAS User's Guide*, 1979) and Duncan's post hoc analysis (Winer, 1971) were used to compare numbers of animals in control and contaminated aquaria ($\alpha = 0.05$).

Chlorpyrifos concentrations were determined with a gas chromatograph equipped with a nitrogen-phosphorus detector. Chlorpyrifos in seawater was extractedd with two 100-ml portions of petroleum ether. The extract was dried by passage through glass wool into a 500-ml Kuderna-Danish concentrator and then evaporated on a steam table to 20 ml, followed by nitrogen evaporation to 2 ml. A control sample fortified with chlorpyrifos (1.0 μg/l) was analyzed concurrently for quality control. The mean recovery for fortified seawater samples exceeded 95%. The lower limit of detection was 0.015 μg/l.

RESULTS

Laboratory-Colonized Communities

The averages and ranges of chlorpyrifos concentrations measured in water from laboratory-developed communities were nondetectable (control), 0.1 μg/l (0.066 to 0.19), 1.2 μg/l (0.74 to 2.1), and 8.5 μg/l (3.1 to 15.0).

A total of 2003 animals representing 29 species of 7 phyla were collected from laboratory-colonized aquaria (Table 1). The chordate *Molgula manhattensis* was most abundant; other dominant phyla were Annelida, Mollusca, Arthropoda, and Coelenterata (an actinarian).

Community structure was affected by all concentration of chlorpyrifos. Average number of species per aquarium significantly decreased (Table 2); a total of 25 species occurred in the control, 19 in 0.1 μg/l, 16 in 1.2 μg/l, and 16 in 8.5 μg/l. The average numbers of individuals and species of mollusks and arthropods per aquarium were significantly decreased by 0.1 μg chlorpyrifos/l and higher concentrations (Table 2). The bittium snail, *Diastoma varium*, and the amphipod, *Corophium acherusicum*, were particularly sensitive and occurred only in control aquaria. Abundance of annelids, coelenterates (an actinarian), and chordates (*Molgula manhattensis*) were not significantly decreased by the highest concentration tested, 8.5 μg/l. However, species of annelids per aquarium were significantly fewer at 8.5 μg/l. Few Echinodermata and Rhynchocoela were collected.

Field-Colonized Communities

The averages and ranges of chlorpyrifos concentrations measured in water from field-developed communities were nondetectable (control),

TABLE 1. Animals in Laboratory-Colonized Benthic Communities Collected from Aquaria[a]

Taxon	Control	Chlorpyrifos treatment (μg/l, for 8 wk)			Total animals
		0.1	1.2	8.5	
Chordata					
Molgula manhattensis	302	356	397	355	1410
Annelida					
Capitella capitata	15	15	15	23	68
Neanthes succinea	13	9	11	7	40
Armandia agilis	6	6	3	3	18
Mediomastus californiensis	3	5	5	5	18
Polydora websteri	5	6	1	2	14
Gyptis vittata	3	5	3	2	13
Cistenides gouldii	1	1	1	0	3
Hydroides protulicola	1	1	1	0	3
Capitomastus aciculatus	2	0	0	0	2
Prionospio cirrifera	0	1	0	1	2
Cirratulus hedgpethi	0	1	0	0	1
Nereis pelagica	0	0	0	1	1
Polydora ligni	1	0	0	0	1
Prionospio cirrobranchiata	1	0	0	0	1
Schistomeringos rudolphi	0	1	0	0	1
Total annelids	51	51	40	44	186
Mollusca					
Acteocina canaliculata	31	15	12	21	79
Mulinia lateralis	16	9	15	11	51
Diastoma varium	16	0	0	0	16
Laevicardium mortoni	7	1	0	1	9
Amygdalum papyrium	3	1	0	1	5
Anadara transversa	1	3	0	0	4
Crassostrea virginica	1	0	1	0	2
Total mollusks	75	29	28	34	166
Arthropoda					
Corophium acherusicum	99	0	0	0	99
Balanus amphitrite	6	13	5	5	29
Anoplodactylus lentus	1	0	1	0	2
Total arthropods	106	13	6	5	130
Coelenterate					
Actinaria	27	24	38	17	106
Echinodermata					
Leptosynapta inhaerens	1	0	1	2	4
Rhynchocoela					
Tetrastemma sp.	1	0	0	0	1
Total All Phyla					
Individuals	563	473	510	457	2003
Species	25	19	16	16	29

[a]Replicates were pooled.

TABLE 2. Average Density of Animals and Number of Species[a] per Laboratory-Colonized Aquarium

Phylum	Control	Chlorpyrifos treatment (μg/l, for 8 wk)		
		0.1	1.2	8.5
Chordata	50.3	59.3	66.2	59.2
	(1.0)	(1.0)	(1.0)	(1.0)
Annelida	8.5	8.5	6.7	7.3
	(5.0)	(4.8)	(3.7)	(3.5[b])
Mollusca	12.5	4.8[b]	4.7[b]	5.7[b]
	(4.3)	(2.3[b])	(1.8[b])	(1.8[b])
Arthropoda	17.7	2.2[b]	1.0[b]	0.8[b]
	(1.8)	(1.0[b])	(0.8[b])	(0.7[b])
Coelenterata	4.5	4.0	6.3	2.8
	(0.8)	(1.0)	(1.0)	(0.7)
Echinodermata	0.2	0	0.2	0.3
	(0.2)	(0)	(0.2)	(0.2)
Rhynchocoela	0.2	0	0	0
	(0.2)	(0)	(0)	(0)
All phyla	93.8	78.8	85.0	76.2
	(13.3)	(10.2[b])	(8.5[b])	(7.8[b])

[a]Given in parentheses.
[b]Significantly different from control at the 5% level.

0.08 μg/l (0.028 to 0.13), 1.0 μg/l (0.93 to 1.16), and 5.9 μg/l (1.5 to 7.9).

A total of 2089 animals representing 65 species of 9 phyla were collected from field-colonized aquaria (Table 3). Annelids were particularly abundant; other dominant phyla were Mollusca, Chordata, Arthropoda, and Echinodermata. Total number of species in control and contaminated aquaria ranged from 40 to 43.

Chlorpyrifos did not significantly decrease total number of individuals or species in field-colonized communities (Table 4). Among phyla, only arthropods were reduced. Numbers and species of arthropods (primarily amphipods) decreased somewhat in 1.0 μg/l and decreased significantly in 5.9 μg/l.

Numbers of the polychaete *Cistenides gouldii* in 1.0 μg/l and in 5.9 μg/l treatments were significantly higher than those in the lowest concentration and in the control. There was no real difference in the total number of other annelids among control and contaminated aquaria. Environmental conditions that were particularly favorable for *C. gouldii* in the higher concentrations may have been in some degree due to toxicant-induced changes in community sttructure, such as reduction of arthropods.

Numbers of individuals and species of mollusks, chordates, and echinoderms in contaminated aquaria did not differ significantly from

TABLE 3. Animals in Field-Colonized Benthic Communities[a]

Taxon	Control	Chlorpyrifos (μg/l, for 7 d) 0.08	1.0	5.9	Total animals
Annelida					
Cistenides gouldii	88	84	250	193	615
Haploscoloplos sp.	79	48	70	65	262
Capitella capitata	47	38	41	26	152
Prionospio heterobranchia	15	10	9	9	43
Glycera americana	9	3	5	6	23
Sabellidae (unid. sp.)	4	5	9	5	23
Neanthes succinea	3	1	3	11	18
Loimia viridis	3	4	3	4	14
Spiophanes bombyx	7	0	2	3	12
Lumbrineris sp.	3	0	4	1	8
Cirratulidae (unid. sp.)	3	0	4	0	7
Poecilochaetus johnsoni	0	2	2	3	7
Gyptis vittata	1	2	0	1	4
Armandia agilis	0	1	0	2	3
Paraonidae (unid. sp.)	0	0	1	2	3
Arenicola cristata	0	1	1	0	2
Phyllodoce sp.	1	1	0	0	2
Polydora sp.	0	0	2	0	2
Sthenelais articulata	1	0	1	0	2
Apoprionospio sp.	0	0	0	1	1
Magelona rosea	0	0	0	1	1
Melinna maculata	0	1	0	0	1
Nephtys sp.	0	0	0	1	1
Paraprionospio pinnata	1	0	0	0	1
Travisia parva	0	0	0	1	1
Terebellid/Ampharetid	1	0	0	0	1
Total annelids	266	201	407	335	1209
Mollusca					
Laevicardium mortoni	45	44	61	69	219
Diastoma varium	17	11	13	11	52
Mitrella lunata	9	7	9	11	36
Tellina sp.	6	5	14	6	31
Lyonsia hyalina	2	1	8	10	21
Marginella apicina	3	4	4	4	15
Crepidula plana	2	1	2	6	11
Nassarius vibex	1	3	0	4	8
Anadara transversa	2	1	2	2	7
Polinices duplicatus	0	0	4	3	7
Mangelia quadrata	1	0	5	0	6
Musculus lateralis	1	4	0	0	5
Anomalocardia auberiana	1	1	1	1	4
Crepidula maculosa	2	2	0	0	4
Mactra fragilis	0	2	0	1	3
Neritina virginea	1	0	2	0	3
Abra sp.	0	2	0	0	2
Acteocina canaliculata	0	0	1	1	2
Ensis minor	1	0	1	0	2
Unid. bivalve	0	0	1	1	2
Gastropteron rubrum	0	0	0	1	1
Thais haemastoma	0	1	0	0	1
Total mollusks	94	89	128	131	442

TABLE 3. Animals in Field-Colonized Benthic Communities[a] (*Continued*)

Taxon	Control	Chlorpyrifos (μg/l, for 7 d) 0.08	1.0	5.9	Total animals
Chordata					
Branchiostoma caribaeum	46	48	41	60	195
Anthropoda					
Acanthohaustorius millsi	31	38	7	0	76
Grandidierella bonnieroides	13	8	12	4	37
Ampelisca abdita	14	7	8	0	29
Tanaidacea (Apseudidae)	3	5	0	0	8
Neopanope texana	2	2	1	0	5
Corophium acherusicum	0	0	1	1	2
Cymadusa compta	0	1	1	0	2
Pagurus annulipes	1	1	0	0	2
Upogebia affinis	2	0	0	0	2
Pycnogonida	0	0	1	0	1
Total arthropods	66	62	31	5	164
Echinodermata					
Leptosynapta inhaerens	12	22	9	18	61
Ophiophragmus filograneus	0	0	1	0	1
Total echinoderms	12	22	10	18	62
Rhynchocoela	3	1	2	2	8
Hemichordata	0	0	3	1	4
Phoronida					
Phoronis architecta	1	0	0	2	3
Coelenterata					
Actinaria	0	2	0	0	2
Total all phyla					
Individuals	488	425	622	554	2089
Species	42	40	43	40	65

[a]Replicates were pooled.

those in the control. Few rhynchocoels, hemichordates, phoronids, and coelenterates were collected.

DISCUSSION

Colonization in field- and laboratory-derived communities resulted in sufficient numbers of benthic macrofauna to allow determination of toxicant effects on a variety of taxa. The two studies provided corroborating data on toxicant effects, as well as data that were peculiar

TABLE 4. Average Density of Animals and Number of Species[a] per Field-Colonized Aquarium

Phylum	Control	Chloropyrifos (μg/l, for 7 d)		
		0.08	1.0	5.9
Annelida	33.2	25.1	50.9[b]	41.9
	(7.0)	(5.5)	(6.6)	(7.1)
Mollusca	11.8	11.1	16.0	16.4
	(5.0)	(5.4)	(5.8)	(6.4)
Chordata	5.8	6.0	5.1	7.5
	(0.9)	(0.9)	(0.6)	(1.0)
Arthropoda	8.2	7.8	3.9	0.6[b]
	(3.2)	(3.1)	(2.1)	(0.6[b])
Echinodermata	1.5	2.8	1.2	2.2
	(0.9)	(0.9)	(0.6)	(0.5)
Rhynchocoela	0.4	0.1	0.2	0.2
	(0.2)	(0.1)	(0.2)	(0.2)
Hemichordata	0	0	0.4	0.1
	(0)	(0)	(0.1)	(0.1)
Phoronida	0.1	0	0	0.2
	(0.1)	(0)	(0)	(0.1)
Coelenterata	0	0.2	0	0
	(0)	(0.2)	(0)	(0)
All phyla	61.0	53.1	77.8	69.2
	(17.4)	(16.1)	(16.1)	(16.1)

[a]Given in parentheses.
[b]Significantly different from control at the 5% level.

to each study. Abundance of arthropods was significantly altered in both laboratory and field situations. Numbers of annelids were not reduced in either study; however, species per aquarium were significantly fewer only in laboratory communities at the highest concentration tested. Mollusks were sensitive to chlorpyrifos only in laboratory-colonized communities. This difference may be due to a long period of toxicant exposure and exposure of early larval forms in laboratory communities, compared to short exposure of later developmental stages in field communities.

Some comparisons can be made between the sensitivity of phylogenetic groups to chlorpyrifos in our studies and the sensitivity in acute tests with single species. The chemical was particularly toxic to crustaceans: to *Corophium acherusicum* at 0.1 μg/l in community studies, and to *Mysidopsis bahia* at 0.035 μg/l in acute tests (Schimmel et al., 1982). Of the chordates, a tunicate and a lancelet were not sensitive to the highest concentrations in our study (8.5 μg/l in laboratory-colonized and 5.9 μg/l in field-colonized communities), while the sensitivity of six fishes ranged from 1.7 to 136 μg/l in acute exposures (Schimmel et al., 1982). The effect on mollusks could not have been predicted from acute testing information. Numbers of molluscan larvae

settling in our laboratory-colonized communities were significantly affected by 0.1 μg/l; however, the acute value for oyster larvae in a static test was much higher, 1991 μg/l (Borthwick and Walsh, 1981). Perhaps exposure methods other than standard acute tests are needed to determine sensitivity of mollusks relative to pesticides.

Of an overall total of 78 species, only 20 (26%) appeared in both field and laboratory communities. However, the more abundant species of Annelida and Mollusca usually were common to both situations. In an earlier study in which colonization of field and laboratory communities occurred simultaneously, 30% of the species were present in both, and the most abundant annelid and mollusk were the same (Tagatz et al., 1981). Similarities between laboratory and field communities in the present study were less likely because the period of colonization and aquarium size differed. In addition, fouling organisms (such as *Molgula manhattensis*) have specific requirements for firm attachment, satisfied by the elevated sides of the laboratory aquaria but possibly not by the soft sediment of the sand-filled boxes.

Our studies and the acute tests cited earlier indicate that concentrations of $\leqslant 0.1$ part per billion of chlorpyrifos in water could be hazardous to saltwater ecosystems by their impacts on individual species, on communities of organisms, and on a wide variety of ecological relationships among benthic and water-column animals.

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